PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

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Applicant's or agent's file reference M80789771:DLT:NAW:ap	FOR FURTHER ACTION	See Form PCT/IPEA/416
International application No. PCT/AU2004/001633	International filing date *(day/month/year)* 24 November 2004	Priority date *(day/month/year)* 24 November 2003

International Patent Classification (IPC) or national classification and IPC

Int. Cl.

C12N 15/29 (2006.01) ***C07K 14/415*** (2006.01)

Applicant

AGRICULTURE VICTORIA SERVICES PTY LTD et al

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 5 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. [X] *(sent to the applicant and to the International Bureau)* a total of 4 sheets, as follows:
      - [ ] sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      - [ ] sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.

   b. [ ] *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or table related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

[X]	Box No. I	Basis of the report
[ ]	Box No. II	Priority
[ ]	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
[ ]	Box No. IV	Lack of unity of invention
[X]	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
[ ]	Box No. VI	Certain documents cited
[ ]	Box No. VII	Certain defects in the international application
[X]	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
26 September 2005	20 February 2006
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer **JAMIE TURNER** Telephone No. (02) 6283

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/AU2004/001633

Box No. I Basis of the report

1. With regard to the **language**, this report is based on:

[X] The international application in the language in which it was filed

[ ] A translation of the international application into , which is the language of a translation furnished for the purposes of:

[ ] international search (under Rules 12.3(a) and 23.1 (b))

[ ] publication of the international application (under Rule 12.4(a))

[ ] international preliminary examination (Rules 55.2(a) and/or 55.3(a))

2. With regard to the **elements** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

[ ] the international application as originally filed/furnished

[X] the description:

pages **1-44** as originally filed/furnished
pages* received by this Authority on with the letter of
pages* received by this Authority on with the letter of

[X] the claims:

pages as originally filed/furnished
pages* as amended (together with any statement) under Article 19
pages* **45-48** received by this Authority on **26 September 2005** with the letter of **26 September 2005**
pages* received by this Authority on with the letter of

[X] the drawings:

pages **1/108 – 108/108** as originally filed/furnished
pages* received by this Authority on with the letter of
pages* received by this Authority on with the letter of

[X] a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing.

3. [X] The amendments have resulted in the cancellation of:

[ ] the description, pages
[X] the claims, Nos. **27, 28**
[ ] the drawings, sheets/figs
[ ] the sequence listing *(specify)*:
[ ] any table(s) related to the sequence listing *(specify)*:

4. [ ] This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond ~~the disclo~~sure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

[ ] the description, pages
[ ] the claims, Nos.
[ ] the drawings, sheets/figs
[ ] the sequence listing *(specify)*:
[ ] any table(s) related to the sequence listing *(specify)*:

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

Box No. V **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-26	YES
	Claims		NO
Inventive step (IS)	Claims	1-26	YES
	Claims		NO
Industrial applicability (IA)	Claims	1-26	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

The following documents are relevant to this international application:

D1 – KUIPER, MJ et al. (2001) Biophysical Journal 81: 3560-5
D2 – PUDNEY, PD et al. (15 February 2003) Archives of Biochemistry and Biophysics 410: 238-45
D3 – EMBL Accession No AJ277399.1 (29 April 2000) SIDEBOTTOM, CM
D4 – WO 2004/022700
D5 – GIDEKEL, M et al. (2 September 2003) Extremophiles 7:459-69

Each of D1-D4 discloses polynucleotide and polypeptide sequences of an antifreeze peptide from Lolium perenne, but no prior sequence discloses nucleotides as shown in Figures 26, 27, 29 and 30. Therefore the claims are novel and inventive in view of any of D1-D4.

While D5 discusses three cold acclimatisation-responsive genes, and their corresponding polypeptides from *Deschampsia antarctica*, D5 does not disclose the antifreeze proteins of the present specification. Therefore the claims referring to *Deschampsia antarctica* are novel and inventive in view of D5

The claimed matter appears to be possess Industrial Applicability.

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

The claims are not fully supported for those claims that specify a "variant thereof". Such a term is broad and largely unsupported except to the extent that the claimed polypeptides have the same biological activity as the regulatory elements and ice recrystallisation protein. This applies to the claimed polynucleotides encoding the same.

Supplemental Box Relating to Sequence Listing

Continuation of Box No. I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established on the basis of:

   a. type of material
      - [X] a sequence listing
      - [ ] table(s) related to the sequence listing

   b. format of material
      - [X] on paper
      - [X] in electronic form

   c. time of filing/furnishing
      - [X] contained in the international application as filed
      - [X] filed together with the international application in electronic form
      - [ ] furnished subsequently to this Authority for the purposes of search and/or examination
      - [ ] received by this Authority as an amendment* on

2. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

* *If item 4 in Box No. I applies, the listing and/or table(s) related thereto, which form part of the basis of the report, may be marked "superseded."*

CLAIMS

1. A substantially purified or isolated nucleic acid or nucleic acid fragment encoding an ice recrystallisation inhibition protein (IRIP) from a *Deschampsia* species, or a functionally active fragment or variant thereof.

2. A nucleic acid or nucleic acid fragment according to claim 1 wherein said *Deschampsia* species is *Deschampsia antarctica*.

3. A nucleic acid or nucleic acid fragment according to claim 1 or 2 including a nucleotide sequence selected from the group consisting of (a) sequence shown in Figures 8, 9, 11, 12, 14, 15, 17, 18, 20, 21, 23 and 24 hereto; (b) complements of the sequences recited in (a); (c) sequences antisense to the sequences recited in (a) and (b); (d) functionally active fragments and variants of the sequences recited in (a), (b) and (c); and (e) RNA sequences corresponding to the sequences recited in (a), (b), (c) and (d).

4. A substantially purified or isolated nucleic acid or nucleic acid fragment encoding an IRIP from a *Festuca* species, or a functionally active fragment or variant thereof.

5. A substantially purified or isolated nucleic acid or nucleic acid fragment encoding an IRIP including a nucleotide sequence selected from the group consisting of (a) sequences shown in Figures 26, 27, 29 and 30 hereto; (b) complements of the sequences recited in (a); (c) sequences antisense to the sequences recited in (a) and (b); (d) functionally active fragments and variants of the sequences recited in (a), (b) and (c); and (e) RNA sequences corresponding to the sequences recited in (a), (b), (c) and (d).

6. A substantially purified or isolated regulatory element from an IRIP nucleic acid from a *Deschampsia* species, or a functionally active fragment or variant thereof.

7. A regulatory element according to claim 6 including a nucleotide sequence selected from the group consisting of (a) sequences shown in Figures 32 and 33 hereto;

(b) complements of the sequences recited in (a); and (c) functionally active fragments and variants of the sequences recited in (a) and (b).

8. A substantially purified or isolated regulatory element from an IRIP nucleic acid from a *Lolium* or *Festuca* species, or a functionally active fragment or variant thereof.

9. A regulatory element according to claim 8 including a nucleotide sequence selected from the group consisting of (a) sequence shown in Figure 34 hereto; (b) complement of the sequence recited in (a) and (c) functionally active fragments and variants of the sequences recited in (a) and (b).

10. A construct including one or more nucleic acids or nucleic acid fragments according to any one of claims 1 to 5.

11. A construct according to claim 10 being a vector and further including one or more promoters and one or more terminators, said nucleic acids or nucleic acid fragments, promoters and terminators being operatively linked.

12. A construct including one or more regulatory elements according to any one of claims 6 to 9.

13. A construct according to claim 12 being a vector and further including one or more further nucleic acid molecules capable of modifying plant response to freezing and/or low temperature stress, and one or more terminators, said regulatory elements, further nucleic acids and terminators being operatively linked.

14. A construct according to claim 13 wherein said further nucleic acid molecule is a nucleic acid or nucleic acid fragment according to any one of claims 1 to 5.

15. A plant cell, plant, plant seed or other plant part, including a construct according to any one of claims 10 to 14.

16. A plant, plant seed or other plant part derived from a plant cell or plant according to claim 15.

17. A method of modifying tolerance of freezing and/or low temperature stress in a plant, said method including introducing into said plant an effective amount of a nucleic acid or nucleic acid fragment according to any one of claims 1 to 5, or a construct according to any one of claims 10 to 14.

18. Use of a nucleic acid or nucleic acid fragment according to any one of claims 1 to 5, and/or nucleotide sequence information thereof, and/or single nucleotide polymorphisms thereof as a molecular genetic marker.

19. A substantially purified or isolated nucleic acid or nucleic acid fragment including a single nucleotide polymorphism (SNP) from a nucleic acid fragment according to any one of claims 1 to 5.

20. A substantially purified or isolated IRIP or IRIP-like polypeptide from a *Deschampsia* species, or a functionally active fragment or variant thereof.

21. A polypeptide according to claim 20 wherein said *Deschampsia* species is *Deschampsia antarctica*.

22. A polypeptide according to claim 20 or 21 including an amino acid sequence selected from the group consisting of sequences shown in Figures 10, 13, 16, 19, 22 and 25 hereto; and functionally active fragments and variants thereof.

23. A substantially purified or isolated IRIP or IRIP-like polypeptide from a *Festuca* species; or a functionally active fragment or variant thereof.

24. A substantially purified or isolated IRIP or IRIP-like polypeptide including an amino acid sequence selected from the group consisting of sequences shown in Figures 28 and 31 hereto; and functionally active fragments and variants thereof.

25. A polypeptide encoded by a nucleic acid or nucleic acid fragment according to any one of claims 1 to 5.

26. A preparation for transforming a plant comprising a nucleic acid or nucleic acid fragment according to any one of claims 1 to 5, or a construct according to any one of claims 10 to 14.